

Microstructural behaviour and gelling characteristics of myosystem protein gels interacting with hydrocolloids

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Abstract

Hydrocolloids were added to blue whiting mince in order to study their distribution in the gel and obtain more information on the mechanism through which these additives act on gel characteristics. The hydrocolloids expanded in cavities of varying morphology. The anionic hydrocolloids were mixed throughout the protein matrix, probably through interaction with the myofibrillar protein. The neutral hydrocolloids were dispersed throughout the matrix but did not interact with it and were located simply by inclusion. The thickening hydrocolloids (locust bean gum, guar gum, xanthan gum, carboxymethylcellulose) formed a mesh of filaments inside the cavities; while the gelling hydrocolloids (carrageenans, alginate) covered or lined the cavity interiors with a continuous structure.

INTRODUCTION

In the manufacture of products based on gelation of myofibrillar protein, additives are commonly used to alter texture and binding properties, and to keep the product stable during storage. These additives act physically and/or chemically, producing structural changes in the protein matrix which depend on their composition, distribution, physical state, volume fraction and interaction with the continuous protein matrix (Okada, 1974; Lanier, 1990; Mills, 1990; Filipi and Lee, 1998).

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There are various different models which seek to explain the structures that can be formed by additives and how they can interact with the muscle protein (Ring and Stainsby, 1982; Tolstoguzov and Braudo, 1983; Aguilera and Kessler, 1989; Ziegler and Foegeding, 1991). The model of Ziegler and Foegeding (1991) explains the possible spatial distribution of gelled protein

with gelling and non-gelling agents in the gel matrix: if the additive does not interact with the gelled myofibrillar protein, it may be that the latter is solubilized or dispersed throughout the interstitial space of the gel matrix; if the additive is associated with the gelified myofibrillar protein, this association may be non-specific at isolated locations. Alternatively, it may be a part of the myofibrillar protein network itself, or it may form a network that cooperates structurally with the protein network, or possibly a combination of the last two in which the additive forms its own network in areas where the myofibrillar protein is weakly connected. This agrees with studies by Gómez-Guillén *et al.* (1996), who found that *iota*-carrageenan formed an independent network that established connections between adjacent structures supporting the principal structure formed by the fish protein; egg white forms a mesh of its own or else is dispersed in lumps about the matrix; and starch is incorporated into the network in granular form or lines the walls of the inclusion cavities to give the appearance of a planar mesh. Other studies (Gómez-Guillén *et al.*, 1998) reported that soy protein, casein and gluten were distributed in clusters of varying size and density.

In order to determine how the hydrocolloids were distributed about the matrix and how they interacted in this gelled protein matrix, two staining procedures were used (one for neutral polysaccharides and the other for anionic polysaccharides) and the characteristics of the different gels occurring with each hydrocolloid were analysed

MATERIALS AND METHODS

Blue whiting (*Micromesistius poutassou* Risso) used in this study was caught off the Cantabrian coast in May. Average size was: 23.42 ± 1.17 cm and average weight: 77.83 ± 12.27 g. The proximate composition (%) was: crude protein 12.34 ± 0.32 , moisture 82.18 ± 0.16 , crude fat 0.47 ± 0.04 and ash 0.63 ± 0.01 (analyses do not show cryoprotectant added) followed analyses described in Pérez-Mateos *et al.* (1997).

Preparation of mince and elaboration of gel were carried out as described previously Pérez-Mateos *et al.* (1997). The hydrocolloids added were: locust bean gum as Viscogum[®] BE, guar gum as FFH-200, xanthan gum as Satiaxane[®] CX90, *iota*-carrageenan Satiagel[®] RPT25, *kappa*-carrageenan as Satiagel[®] RPT8, sodium carboxymethylcellulose (CMC) as Tylopur[®] C10.000 or sodium alginate as Satialgine[®] S1100 (SKW Biosystems, Rubí, Barcelona, Spain) at 0.5 % final value (1 % in the case of locust bean gum) and salts (NaCl, KCl) according to results of Pérez-Mateos (1998), with crushed ice to give the required final gel moisture (80 %).

Folding test, puncture test (breaking deformation, breaking force, work of penetration), Texture Profile Analysis (hardness, adhesiveness, cohesiveness) and stress-relaxation test (elasticity), colour (L^* , a^* , b^*) and water holding capacity were determined as Pérez - Mateos *et al.* (1997) but in our case it was decided to compress sample to 60 %.

Light microscopy. Samples were fixed in 10% formaldehyde, dried in increasing series of ethanol (50, 70, 96, 100 °) and toluene and included in paraffin at 56-60 °C. The blocks of paraffin were then cut in 8 μ m-thick sections using a 1130/Biocut microtome (Reichert-Jung, Germany)

and fixed on plates coated with a 1:1 solution of albumin and glycerine. The sections were then hydrated by successive addition of xylol and ethanol in decreasing dilutions (100, 96, 70 °). Gels with *neutral hydrocolloids* were stained with Schiff's reagent (Hotschkiss, 1948; Martoja and Martoja-Pierson, 1970); gels containing *anionic hydrocolloids* were stained with alcian blue
5 (Martoja and Martoja-Pierson, 1970). The control gel (hydrocolloid-free) was also used as control for both types of staining. Picrocarmine was used as contrast colouring in all cases. Finally, all the sections were dried in increasing concentrations of ethanol (70, 96, 100 °) and xylol. Photomicrographs were taken with a light microscope (Nikon Optiphot, model AFX-IIA, Japan) at 100 and 1000 magnifications.

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Scanning electron microscope. Cubes of 2 to 3 mm were cut from inside the gels for microscopic examination and rapidly quenching them to the temperature of liquid nitrogen. The characteristic emission of sulphur under electron bombardment was determined by scanning electron microscope (Jeol Scanning Microscope, JSM 6400, Japan) at 20 kV.

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Statistical analysis. One-way analysis of variance was carried out using the BMP computer programme (BMDP Statistical Software, Inc., Cork Technology Park, Cork, Ireland) to establish differences in gel characteristics induced by the type of hydrocolloid used. Differences of means between pairs between each sample and the hydrocolloid-free gel were determined by Dunnet
20 test (BMDP 7D). Level of significance was set for $p \leq 0.05$.

RESULTS AND DISCUSSION

Examination of the gel structure of sample made from blue whiting muscle with no added

hydrocolloids (Fig.1.A y B) revealed a homogeneous, slightly porous matrix, which was free of cavities or differently-coloured areas because it contained no polysaccharides. The sorbitol added to preserve the minced muscle (4 %) was not detected, probably because the monosaccharides were removed by washing during the staining process.

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In figure 1.C locust bean gum can be seen largely located inside large round cavities evenly distributed throughout the matrix. The fact that the photomicrograph of the hydrocolloid-free gel shows no cavities (Fig.1.A) suggests that these are produced by swelling of the hydrocolloid in the protein matrix; this in turn would account for the fact that the stained surface as a percentage of
10 total matrix surface was higher than the concentration of added hydrocolloid in the formulation.

The locust bean gum is arranged as filaments inside the cavities (Fig.1.D); there appear to be no areas of interaction or contact between the hydrocolloid and the protein matrix. The presence of locust bean gum in the cavities caused a considerable increase of breaking deformation and work of penetration with respect to the hydrocolloid-free gel (Table 1), which suggests that, like many
15 starches, the gum holds water and makes the gel more deformable. However, there were changes in the properties determined by compression test, as the hydrocolloid was very much localized at certain points in the matrix. The fact that the percentage of area occupied by the cavities was higher than the percentage of added hydrocolloid in the formulation (1 %) could be explained by swelling due to retention of water. The only change in colour was a slight but
20 significant increase in redness (a^*) values (Table 1).

Figure 1.E shows guar gum distributed in the protein matrix in much the same way as locust bean gum, probably because both gums are galactomannans. However, there is a smaller number of stained cavities, probably because the concentration was only half (0.5 %). At higher

magnification (Fig. 1.F), the filament arrangement in the cavities is visible. This gel structure had significantly less firmness (breaking force, work of penetration, hardness) than the control (Table 1) but had greater water holding capacity (Table 1). There were no significant changes in gel colour (Table 1).

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Figure 1.G shows xanthan gum in a mesh arrangement inside the distributed cavities. These cavities differ from the ones described above in that they vary more in size, some being relatively large and more irregular. There are few areas of contact with the protein matrix. The presence of xanthan gum produced a decrease in the gel forming capacity of the myofibrillar protein, which
10 was reflected in poorer resistance to the folding test and lower values of most of the other properties analysed (Table 1). This was due on the one hand to the gum's presence in the interstitial spaces of the matrix (Fig.1.H), where its high molecular weight probably hindered formation of the protein network, and on the other hand to the presence of large cavities in the matrix. There were no significant changes in water holding properties or colour (Table 1). It should
15 be remembered that when xanthan gum is in dry state or has little available water, it tends to aggregate and coil upon itself, thus occupying a large volume which would distort the protein matrix.

Staining of the protein gels containing *iota*-carrageenan and *kappa*-carrageenan highlights the
20 distribution of both hydrocolloids in cavities intensely coloured by alcian blue, dispersed throughout the matrix in numerous small elongated cavities (*iota*-carrageenan, Fig. 1.I) or large round cavities (*kappa*-carrageenan, Fig. 1.K). Unlike the other hydrocolloids, there is no filamentous deposition and the cavities are smaller, possibly because these sulphated polysaccharides had gelled, filling the entire cavity. Ultrastructure studies (Gómez-Guillén *et al.*,

1996) suggest that *iota*-carrageenan forms a fine three-dimensional network with some points of connection with the protein matrix. In addition there is considerable continuity of the protein matrix and the hydrocolloid all over the cavity surface, and also dispersed through the matrix (Figs.1.J and 1.L), most apparent in the case of *iota*-carrageenan. This could indicate some chemical
5 interaction between them, probably due to formation of stable bonds during gelation depending on the number of sulphate groups in the hydrocolloid. According to Llanto *et al.* (1990), the increased gel forming capacity of Alaska pollack *suirmi* when carrageenan is added is due to the interaction of the carrageenan's sulphate groups with the myofibrillar protein.

10 Carrageenans are sulphated polysaccharides, and therefore the sulphur can be used to identify the distribution of the carrageenan in the gel. The sulphur map (not shown) of the blue whiting muscle gel indicates that the carrageenan is evenly distributed in the gel. This agrees with studies carried out by Belton *et al.* (1985, 1986).

15 Our results are therefore in reasonable agreement with reports on pork sausages by Trius *et al.* (1994, 1995). According to them, the reason for the different distribution is that *kappa*-carrageenan solubilizes at 60-70 °C, when the myosin has already begun to gel, whereas *iota*-carrageenan solubilizes at 50 °C before the myofibrillar protein has gelled and is thus better able to penetrate the protein matrix. Other researchers (Bater *et al.*, 1992) have reported *kappa*-
20 carrageenan distributed in long thin bands in oven-roasted turkey breast.

The gels containing *iota*-carrageenan had particularly high values of adhesiveness and elasticity but evidenced deterioration of the properties measured by puncture test (Table 1), suggesting that its high level of dispersal probably detracted from gel flexibility. In experiments carried out on

muscle of squid (*Dosidicus gigas*) with and without 2 % added *iota*-carrageenan, Gómez-Guillén and Montero (1997) also found a decrease in breaking deformation of gels containing carrageenan, although they detected no significant differences in breaking force values. On the other hand, Montero *et al.* (1992) and Nakayama *et al.* (1988) reported increased work of penetration in sardine muscle mince gels with added *iota*-carrageenan. In the present case water holding capacity also increased (Table 1); this could be the reason why although only representing 0.5 g per 100 g of total gel mass, the percentage of gelled *iota*-carrageenan with respect to the protein matrix was apparently high. Other authors have also reported increased water holding capacity with addition of *iota*-carrageenan in beef gels (Foegeding and Ramsey, 1987) and giant squid (*Dosidicus gigas*) gels (Gómez-Guillén and Montero, 1997).

In the gels containing *kappa*-carrageenan hardness and adhesiveness increased considerably (Table 1) with respect to the hydrocolloid-free control; in other words, the addition of *kappa*-carrageenan enhanced the gel's binding properties, although there was loss of cohesiveness. Foegeding and Ramsey (1987) also found that *kappa*-carrageenan more effectively increased the hardness of beef gels than *iota*-carrageenan. Niwa *et al.* (1988) reported high adhesive strength between the *kappa*-carrageenan suspension and the surface in Alaska pollack *suirmi* gels. Addition of this hydrocolloid did not increase water holding capacity with respect to the control (Table 1). According to da Ponte *et al.* (1985), *iota*-carrageenan has greater water holding capacity than *kappa*-carrageenan and further prevents syneresis during thawing of frozen fish gels. On the other hand DeFreitas *et al.* (1997) found that both carrageenans increased the water holding capacity of soluble pork proteins. These differences in the effect of one and the same hydrocolloid may be the result of both intrinsic characteristics and processing conditions.

Figure 1.M shows sodium carboxymethylcellulose in small cavities throughout the protein matrix. This is possibly because CMC particularly requires a lot of water in order to disperse, a condition not favoured by addition in dry state (Keller, 1986). The number of stained cavities is smaller than in all other cases, possibly because the CMC had hardly trapped any water as the water holding capacity of the gel was not augmented. However, this gel also presented low values for the rheological properties determined by puncture test (Table 1), although it is the structure that most closely resembles the photomicrograph of the control gel (Fig.1.A). Thus, no relationship was apparent between the hydrocolloid concentration and the mechanical characteristics of the gel. The fact that it did not expand could explain why there is no change of appearance in the photomicrograph, the hydrocolloid's elongated shape ensuring that it is distributed through the matrix in small strands and is barely visible. However, its presence did modify the gel rheology, probably through interaction of its carboxyl groups with the matrix, and because the matrix was distorted by its high molecular weight. As in other cases, it can be seen inside the cavity as alcian blue filamentous mesh; in addition, only a small part is bonded to the matrix (Fig.1.N). Other authors (Barbut and Mittal, 1996) have reported that addition of CMC (0.5 %) reduced the WHC of frankfurters, probably because the CMC enveloped the myofibrillar protein. The same authors also found that the textural characteristics of the product were not affected at so low a concentration. In the present case, CMC caused significant increases in values of redness a^* and yellowness b^* (Table 1). On the other hand Barbut and Mittal (1996) found that lightness decreased when 0.5 % CMC was added to frankfurters.

In the gels containing sodium alginate the hydrocolloid, stained alcian blue, can be seen inside relatively small elongated crack-like cavities (Fig.1.O), possibly due to its elongated molecular structure. At higher magnifications (Fig.1.P) areas are visible where the alginate is connected to

the protein matrix, although not completely as with the carrageenans and without the fibrous appearance of the non-gelling hydrocolloids. As in the case of *kappa*-carrageenan, alginate produced blue whiting gels with high hardness and adhesiveness values, although the values of all the other characteristics analysed were lower than in the control (Table 1). As regards alterations in colour, the gel presented greater redness a^* and yellowness b^* (Table 1) the same as was found with carboxymethylcellulose.

In summary, the addition of hydrocolloid significantly reduced puncture test properties with respect to the hydrocolloid-free gels. This would suggest that in the given conditions the additives did not greatly reinforce the protein matrix because the mince used had good gel forming capacity. On the other hand, compression properties were altered to a greater or lesser extent in gels containing anionic hydrocolloids, coinciding with their presence in the matrix and not only in the cavities, probably due to chemical interaction with the myofibrillar protein. In this connection, some authors (Lee and Chung, 1989; Gómez-Guillén *et al.*, 1997) noted that while penetration test measures the degree of compactness or density of actomyosin, compression test measures the overall binding properties of gel material.

In contrast, in most cases the addition of hydrocolloids enhanced water holding properties (Table 1). This increase is directly attributable to the individual hydrocolloid and to the fact that the matrix is formed by protein/hydrocolloid interaction. There were no notable alteration in colour (Table 1), because the hydrocolloid was added at a very low concentration.

As regards distributions, the gelling hydrocolloids (carrageenans and alginates) totally occupied the cavity, whereas the thickening hydrocolloids (locust bean gum, guar gum, xanthan gum,

carboxymethylcellulose) formed a mesh of filaments inside the cavities. Moreover, the anionic hydrocolloids were spread through the matrix, although less so in the case of CMC, whose addition in dry state does not favour interaction with the medium. This is in agreement with a previous experiment (Pérez-Mateos, 1998) where cluster analysis showed that the gels containing hydrocolloids appeared to possess similar functionality according to the electric charge, chemical composition and molecular size of the polysaccharide: that is, the gels containing neutral hydrocolloids, and the gels containing anionic hydrocolloids. Of the latter, xanthan gum is a case apart, probably due to its high molecular weight.

10 It is important to note that as the hydrocolloid is added in dry state, the amount of water in the batter is mainly retained by the myofibrillar protein and hence the hydrocolloid only entraps what water is released by the protein.

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